IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Richard F Selden et al.
Serial No. : 09/686,497
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Title : OPTIMIZED MESSENGER RNA

Art Unit : 1652
Examiner : N. Nashed

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

BRIEF ON APPEAL

(1) Real Party in Interest

The Real Party in Interest is Transkaryotic Therapies, Inc., 700 Main Street, Cambridge, MA 02139.

(2) Related Appeals and Interferences

There are no pending related appeals or interferences.

(3) Status of Claims

Claims 15-25 are canceled.

Claims 1-14 and 26-32 are rejected under 35 U.S.C. §103 and are under appeal. Claims 1-14 and 26-32 are also provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Application No. 09/407,605. As noted in the reply filed October 22, 2003, a terminal disclaimer to overcome the provisional rejection of the claims under the judicially created doctrine of obviousness-type double patenting will be filed upon notification of allowance. As such, these rejections are not addressed in this brief.

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(4) Status of Amendments

All of the amendments filed in this case have been entered. No amendments are being submitted herewith.

(5) Summary of Invention

The invention relates to a synthetic nucleic acid sequence that encodes human α-galactosidase, wherein at least one non-common codon or less-common codon has been replaced by a common codon and wherein the synthetic nucleic acid has one or more of the following properties: (a) it has a continuous stretch of at least 150 codons, e.g., at least 200, 250, or 300 codons, all of which are common codons; (b) it has a continuous stretch of common codons that comprises at least 60%, e.g., at least 70%, 80%, 90%, or 95%, of the codons of the synthetic nucleic acid sequence; and (c) at least 94%, e.g., at least 96%, 98%, or all, of the codons in the sequence encoding the protein are common codons. The number of non-common or less-common codons remaining in the synthetic nucleic acid sequence can be less than 15, e.g., 0, or can be equal or less than 6% of the codons in the synthetic nucleic acid sequence. The synthetic nucleic acid sequence can be inserted into a non-transformed cell. A vector or a cell can comprise the synthetic nucleic acid sequence. See, e.g., page 12, line 5, to page 14, line 12; and page 27, lines 13-20.

The invention also relates to a method of producing α-galactosidase comprising culturing a cell comprising the synthetic nucleic acid sequence under conditions in which the nucleic acid is expressed. See, e.g., page 51, lines 8-14; and page 72, lines 4-6, and Table 10.

The invention also relates to a method for preparing a synthetic nucleic acid sequence encoding human α-galactosidase, comprising identifying a non-common codon and a less-common codon in a non-optimized gene sequence which encodes an α-galactosidase protein, and replacing at least 94%, e.g., at least 96% or 98%, of the non-common and less-common codons with a common codon encoding the same amino acid as the replaced codon. See, e.g., page 14, lines 14-20.

(6) Issue

Are claims 1-14 and 26-32 obvious based on 35 U.S.C. § 103(a) over Seed WO96/09378 (Seed) in view of Kim et al. 1997, *Gene* 199:293-301 (Kim); Morgan et al., *Pediatr. Nephrol.* 1:536-539 (Morgan); Bishop et al., 1986, *PNAS USA* 83:4859-63 (Bishop); and Wada et al., 1992, *Nucleic Acids Res.* 20:2111-18.

(7) Grouping of Claims

The claims should stand or fall together.

(8) Argument

In view of the lack of motivation to arrive at the invention, the clear teaching-away from the invention found in Seed and Kim, and Appellants' surprising results, claims 1-14 and 26-32 are not obvious over Seed in view of Kim, Morgan, Bishop, and Wada.

Claims 1-14 and 26-32 stand finally rejected under 35 U.S.C. § 103(a) as being obvious over Seed WO96/09378 (Seed) in view of Kim et al. 1997, *Gene* 199:293-301 (Kim); Morgan et al., *Pediatr. Nephrol.* 1:536-539 (Morgan); Bishop et al., 1986, *PNAS USA* 83:4859-63 (Bishop); and Wada et al., 1992, *Nucleic Acids Res.* 20:2111-18 (Wada).

In the Advisory Action mailed November 25, 2003 ("Advisory Action"), the Examiner stated that the claims were rejected under 35 U.S.C. § 103(a) as being unpatentable, as outlined above, "for the reasons set forth in the prior Office action, paper number 13" (Advisory Action, page 3). In paper number 13 (the Office Action mailed November 8, 2002, hereinafter "First Office Action"), the Examiner stated that Morgan provides the motivation "to develop a method for making α-galactosidase to use for the treatment of Anderson-Fabry disease" (at page 5). The Examiner also asserts that Seed and Kim provide motivation "to synthesize α-galactosidase in which the non-common and less common codons are replaced with common codon [*sic*] for mammalian cells to enhance the expression of α-galactosidase A in said cells" (First Office Action, page 5). According to the Examiner,

> it would have been obvious to one of ordinary skill in the art to identify the less common and non-common codon [*sic.*] used for mammalian cells in a gene

> encoding α-galactosidase such as the human α-galactosidase A taught by Bishop *et al.*, synthesize the codon optimized gene, construct a mammalian expression vectors [*sic.*] comprising the synthetic gene, transform a mammalian cell with said vector, and express the synthetic gene in a mammalian cell of choice as taught by Seed and Kim *et al.* to make the desired α-galactosidase....Also, applicants should note that any α-galactosidase gene from any biological source can be optimized for express in almost any host cell including human, *E. coli*, yeast, and insect among others because the common codons for many organisms are known, see Wada *et al.* (First Office Action, page 5).

Appellants respectfully traverse this rejection.

The Examiner's basis for the rejection is that the art provides a motivation to generally optimize genes. This is not the issue, as the claims are not directed to "optimized" genes but rather to sequences having specific and very high levels of optimization. The issue is whether the combination of references suggests optimization to **the very high levels required by the claims**. As is discussed in more detail below, the references, singly and in combination, fail to suggest any gene, much less the specifically claimed gene (human α-Gal), having the high levels of common codons required by the claims. In addition, as discussed in detail below, the references provide a pattern of teaching away from such high levels of optimization of a human gene. Furthermore, the nucleic acids of the claims have surprising and unexpected properties. For all of these reasons the claims are not obvious over the art.

Seed

Seed teaches a synthetic gene encoding a protein normally expressed in mammalian cells wherein at least one non-preferred or less preferred codon in the natural gene encoding the mammalian protein has been replaced by a preferred codon encoding the same amino acid (see Abstract). However, Seed provides only a generalized description of optimized nucleic acid sequences that neither teaches nor suggests synthetic sequences (much less α-galactosidase sequences) with the very high level or range of common codons required by the claims. In fact Seed teaches away from the claimed invention. Seed provides explicitly that

> [in] constructing the synthetic genes of the invention it may be desirable to avoid CpG sequences as these sequences may cause gene silencing" (at page 4, lines 10-12, emphasis added).

Seed thereby cautions against and leads away from substantial increased use of CpG pairs. (The use of common codons of the claims increases the number of CpG pairs.) This is acknowledged by the Examiner at page 4 of the Office Action mailed May 27, 2003 ("Final Office Action"). Given the teachings of Seed, such a modification, at the time the application was filed, would have been predicted to cause gene silencing, as is explicitly stated in Seed (see the passage quoted above). Thus, one of skill in the art would not have been motivated to make the nucleic acids of the invention and furthermore would have been discouraged from doing so. One of ordinary skill would have been expressly discouraged by Seed from replacing the codons of synthetic genes with human common codons if the replacement resulted in a substantial increase in CpG pairs. This teaches away from the invention.

According to the Federal Circuit:

> [a] reference may be said to teach away when a person of ordinary skill in the art, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant...(Tec Air, Inc. v. Denso Mfg. Michigan Inc., 192 F.3d 1353, 1360 (Fed. Cir. 1999), emphasis added).

The Examiner was not convinced that Seed teaches away. The Examiner interpreted Seed as follows, "Although Seed caution of the increased use of CpG he it did not consider it *fatal* in reengineering any gene" (Final Office Action, page 4, emphasis added). However, the law does not require that a teaching away be "fatal". The Federal Circuit has stated that "[a] *prima facie* case of obviousness can be rebutted if the applicant...can show 'that the art in *any* material respect taught away' from the claimed invention" (In re Haruna, 249 F.3d 1327, 1335 (Fed. Cir. 2001), emphasis added).

Seed, in a very material way, teaches away from the claimed invention, namely that high levels of CpG sequences (and thus high levels of common codons) should be avoided. Thus, the Examiner's argument that Seed does not teach that the use of the CpG sequences is fatal does not have a valid legal basis and Appellants have met the burden of showing that Seed teaches away from the invention in a material way.

In the Advisory Action, the Examiner maintains that "the cautionary note provided by Seed does not teach away from the claimed invention because the optimized gene encoding HIV envelope [*sic.*] glycoprotein...displays enhanced expression in mammalian cells" (Advisory Action, page 4). This argument is not germane to the present claims. The HIV envelope glycoprotein is not a human sequence. Rather, the passage quoted by the Examiner refers to changing **non-human** sequences (HIV envelope glycoproteins and jellyfish GFP) to match the codons prevalent in human sequences. In contrast, the claims recite replacing non-preferred codons in a particular **human** sequence (one encoding human alpha-Gal A) with common **human** codons. This is a completely different concept than replacing codons in a non-human sequence with common human codons.

In the office action dated May 27, 2003, the Examiner argued that the level of common codons required by the claims do not confer patentability. Relying on the fact that naturally occurring human common codons have a tendency to favor CG pairs the Examiner argued, "the human species has not vanished because of its preference for codons having the CG pairs". Applicants agree that native human sequences perform well in the context in which they evolved. However, the native human α-galactosidase sequence has a much lower level of human preferred codons than the synthetic sequence recited in the claims. Thus, the optimized functionality of the native human sequence, to which the Examiner refers, relies on codon usage much different than does the claimed invention. That is, the claims require a much higher level of human common codons than the native α-galactosidase sequence, which Applicants agree works well in nature. The claims tell a skilled artisan to take a human sequence that has already been optimized by nature (human alpha-Gal A) and modify it by replacing the non-common codons with common codons at the very high levels specifically recited. Seed provides no motivation to a skilled person to do this. Surprisingly, Applicants found that synthetic α-galactosidase sequences containing the much larger continuous stretches or overall very high numbers of common codons recited in the claims, resulted in 2.0- and 5.7-fold increases in mean α-galactosidase expression compared to the wild-type sequence (see table 10 and accompanying discussion at page 72 of Applicants' disclosure). These are surprising results, especially in view of the teaching away by Seed and Kim.

In summary, while Seed generally teaches the optimization of nucleic acid sequences, nothing in that reference teaches or suggests optimizing a human sequence to the very high levels specified in the claims. In fact, Seed teaches away from optimizing at the recited levels by cautioning that CpG sequence (which inevitably result form the use of high levels of common codons) can have detrimental effects and suggesting that such sequences be avoided. The Examiner ignores this statement, arguing that Seed shows enhanced expression in mammalian cells using an optimized gene encoding the HIV envelope glycoprotein. However, as pointed out by the Appellants, the Examiner's argument is not germane to the claimed invention. HIV envelope glycoprotein (or GFP) is not a human sequence. Appellants have taken a human sequence that has already been optimized by nature and modified it to replace the non-common and less-common codons with common codons at very high levels and without regard for the CG content. It view of the teachings in the art such as Seed and the fact that the sequence chosen was one already produced in humans, it was surprising that optimization at the high levels recited in the claims resulted in enhanced expression of human alpha-Gal A.

Kim

As discussed herein, Appellants' claims are directed to synthetic α-galactosidase nucleic acids (and related methods of making them) that have one or more of: a very long stretch of common codons (at least 150 continuous common codons); a stretch of common codons that is a very large percentage of the total sequence (at least 60% of the total sequence is common codons); or a very high percentage of common codons (at least 94% common codons).

According to the Examiner,

> Kim *et al.* teach that selective codons in a given gene positively correlate with its expression efficiency, see abstract. Also, they teach the codon optimization of a leader sequence leads to further enhancement of a synthetic gene, see page 297, right column, section 3.3 (First Office Action, page 5).

Appellants respectfully disagree with this characterization. Kim does teach that the expression of human erythropoietin (EPO) can be increased by replacing some less-common codons with high-frequency codons (which is essentially the same as a common codon). However, Kim

found that a yeast/human hybrid EPO gene yielded very high expression. The Examiner asserts that "codon optimization of a leader sequence [that] leads to further enhancement". However, the Kim reference does not refer to optimization with human high-frequency codons. Instead, the hybrid EPO gene described by Kim contained yeast high-frequency codons at the 5'leader sequence and at the sequence encoding the first six amino acids of mature EPO, and human high-frequency codons throughout most, but not all, of the rest of the EPO hybrid gene (see Fig. 1 of Kim and Table 1 of the present specification). Thus, Kim neither teaches, nor suggests, any sequence, much less a synthetic α-galactosidase nucleotide sequence, containing the very large continuous stretches or overall very high numbers of common codons recited in the claims.

Further, despite the Examiner's characterization of Kim, that reference, by cautioning against the overuse of human common codons, continues the pattern of teaching away seen in the above-discussed Seed reference.. In the Advisory Action, the Examiner asserted that:

> Kim...writes: 'Reengineering the coding sequence to match to the codons frequently found in human genes is beneficial to achieve high-level of expression. Recent reports clearly support this. Altering the coding sequence of the HIV envelope glycoprotein gp120 and jellyfish green fluorescent protein gene to human prevalent codons results in substantial increase in efficiency (Haas *et al.* 1996; and Zolotukhin *et al.* 1996.' (See Kim *et al.*, the last paragraph on page 299). *Although Seed has a cautionary remark regarding the over use of human preferred codon* [*sic.*] *containing CpG, Kim et al. have none* (Advisory Action, page 4, emphasis added).

This is not the entire picture. Kim follows the sentences quoted above by stating unequivocally that:

> [r]e-engineered genes with human codon usage become high in their GC content. Although a low GC content of 5' UTR is ensured, optimizing the re-engineered gene further by *decreasing the GC content* of the limited region downstream of the initiator codon *is advisable* (Kim, page 299, last paragraph, emphasis added).

Thus, Kim does caution away from the overuse of human common codons, particularly downstream of the initiator codon. Far from decreasing the GC content of any region of the synthetic sequence of taught by Kim, Appellants' presently claimed synthetic sequences have substantially increased GC content compared to the non-optimized sequence, regardless of the region of the sequence.

Further, the EPO^h coding sequence of Kim includes at least the following amino acids encoded by non-common human codons: P2, P3, R4, L5, R14, C33, P87, P121, P122, and A124. For example, the most common codon encoding proline (P) in highly expressed human genes is CCC (see Fig. 1 of Kim and Table 1 of the present specification). However, the EPO coding sequence shown in Fig. 2 of Kim uses the codons CCA (P2, P3 and P87) and CCG (P121 and P122). Kim states that "[s]ome deviations from strict adherence to prevalent codon usage were made to accommodate the introduction of unique restriction sites or to avoid homopolymeric DNA sequences" (Kim, page 297, first column). Kim thus teaches away from replacing codons with human common codons where the resulting sequence would contain repeated consecutive nucleotides.

Upon reading the disclosure of Kim, one of skill in the art would be discouraged from (1) replacing sequences within the region downstream of the initiator codon with human (CG-rich) common codons, and (2) overusing human common codons where strict adherence to common codon usage would result in a high level of GC sequences. The claimed nucleic acids go against these cautionary paths. Kim clearly teaches away from the claimed invention.

Thus, Seed and Kim, individually, or in combination, fail to suggest the nucleic acids of the claims and in fact both teach away from such nucleic acids. The other references relied on by the Examiner, namely Morgan, Bishop and Wada, fail to add anything, and specifically fail to suggest the use the high levels of common codons required by the claims. Thus, alone, or combined with the other references, they fail to remedy the shortcomings of Seed and/or Kim. Furthermore, nothing in the remaining references alters the pattern of teaching away seen in Seed (the primary reference) and Kim.

The Teachings of Morgan, Bishop and Wada

With Seed and Kim failing to suggest the invention and further, actually teaching away from the present claims, the Examiner is left only with Morgan, Bishop and Wada to provide the motivation and reasonable expectation of success required for a *prima facie* case of obviousness. Morgan teaches that Anderson-Fabry disease is an X-linked lysosomal storage disorder due to α-galactosidase A deficiency but provides no suggestion to use the high levels of common codons

required by the claims. Bishop describes the human α-galactosidase nucleotide sequence, and that mutations at the α-galactosidase A locus of the X chromosome result in Fabry disease but provides no suggestion to use the high levels of common codons required by the claims. Wada describes the frequency of codon use in different genes of different organisms but provides no suggestion to use the high levels of common codons required by the claims.

Morgan, Bishop, and Wada, fail to add anything which would remedy the shortcomings of Seed and/or Kim and suggest the nucleic acids of the invention. Furthermore, nothing in the remaining references alters the pattern of teaching away seen in Seed (the primary reference) and Kim.

Conclusion

Appellants have found that expression of human α-galactosidase can be enhanced by the construction of human α-galactosidase synthetic sequences in which a very high percentage of non-common or less-common codons is replaced with human common codons. As discussed above, the references relied on by the Examiner, singly, or in combination, fail to suggest the use of the high levels of common codons required by the claims . Furthermore, both Seed and Kim teach away from the subject matter of the claimed invention. Contrary to the teachings of Seed and Kim, Appellants have provided a fruitful strategy for increased protein expression with disregard to CpG content. (See Appellants' disclosure at page 43, lines 25-28). Indeed, Appellants found that synthetic α-galactosidase sequences containing the very large continuous stretches or overall very high numbers of common codons recited in the claims, resulted in 2.0- and 5.7-fold increases in mean α-galactosidase expression compared to the wild-type sequence (see table 10 and accompanying discussion at page 72 of Appellants' disclosure). .

Therefore, none of the cited references, alone or in combination, teach or suggest all elements of the present claims. Even if a prima facie case of obviousness were made, Appellants' surprising results would overcome it. As such, claims 1-14 and 26-32 are not obvious based on 35 U.S.C. § 103(a) over Seed in view of Kim, Morgan, Bishop and Wada.

Appellants have enclosed the brief fee of $165. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 6/28/04

Laurie Butler Lawrence
Reg. No. 46,593

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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The PTO did not receive the following listed item(s) ____

Appendix of Claims

1. A synthetic nucleic acid sequence that encodes human α-galactosidase, wherein at least one non-common codon or less-common codon has been replaced by a common codon and wherein the synthetic nucleic acid has one or more of the following properties:

it has a continuous stretch of at least 150 codons all of which are common codons;

it has a continuous stretch of common codons, which continuous stretch comprises at least 60% of the codons of the synthetic nucleic acid sequence;

at least 94% of the codons in the sequence encoding the protein are common codons, wherein by a common codon is meant Ala (gcc); Arg (cgc); Asn (aac); Asp (gac); Cys (tgc); Gln (cag); Gly (ggc); His (cac); Ile (atc); Leu (ctg); Lys (aag); Pro (ccc); Phe (ttc); Ser (agc); Thr (acc); Tyr (tac); Glu (gag); Val (gtg), Met (atg) and Trp (tgg).

2. The synthetic nucleic acid sequence of claim 1, where the α-galactosidase nucleic acid is inserted into a non-transformed cell.

3. The synthetic nucleic acid sequence of claim 1, wherein the number of non-common or less- common codons remaining is less than 15.

4. The synthetic nucleic acid sequence of claim 1, wherein the number of non-common or less- common codons remaining, taken together, are equal or less than 6% of the codons in the synthetic nucleic acid sequence.

5. The synthetic nucleic acid sequence of claim 1, wherein all non- common or less-common codons are replaced with common codons.

6. The synthetic nucleic acid sequence of claim 1, wherein at least 96% of the codons in the synthetic nucleic acid sequence are common codons.

7. The synthetic nucleic acid sequence of claim 1, wherein at least 98% of the codons in the synthetic nucleic acid sequence are common codons.

8. The synthetic nucleic acid sequence of claim 1, wherein all the codons of the synthetic nucleic acid sequence are common codons.

9. A vector comprising the synthetic nucleic acid sequence of claim 1.

10. A cell comprising the nucleic acid sequence of claim 1.

11. A method of producing α-galactosidase comprising culturing the cell of claim 10 under conditions in which the nucleic acid is expressed.

12. A method for preparing a synthetic nucleic acid sequence encoding human α-galactosidase, comprising:

identifying a non-common codon and a less-common codon in a non-optimized gene sequence which encodes an α-galactosidase protein; and

replacing at least 94% of the non-common and less-common codons with a common codon encoding the same amino acid as the replaced codon,

wherein by a common codon is meant: Ala (gcc); Arg (cgc); Asn (aac); Asp (gac); Cys (tgc); Gln (cag); Gly (ggc); His (cac); Ile (atc); Leu (ctg); Lys (aag); Pro (ccc); Phe (ttc); Ser (agc); Thr (acc); Tyr (tac); Glu (gag); Val (gtg), Met (atg) and Trp (tgg).

13. The method of claim 12, wherein at least 96% of the non-common and less-common codons are replaced with a common codon encoding the same amino acid as the replaced codon.

14. The method of claim 12, wherein at least 98% of the non-common and less-common codons are replaced with a common codon encoding the same amino acid as the replaced codon.

26. The synthetic nucleic acid sequence of claim 1, wherein the nucleic acid has a continuous stretch of at least 200 common codons.

27. The synthetic nucleic acid sequence of claim 1, wherein the nucleic acid has a continuous stretch of at least 250 common codons.

28. The synthetic nucleic acid sequence of claim 1, wherein the nucleic acid has a continuous stretch of at least 300 common codons.

29. The synthetic nucleic acid sequence of claim 1, wherein the nucleic acid has a continuous stretch of common codons, which continuous stretch comprises at least 70% of the codons of the synthetic nucleic acid sequence.

30. The synthetic nucleic acid sequence of claim 1, wherein the nucleic acid has a continuous stretch of common codons, which continuous stretch comprises at least 80% of the codons of the synthetic nucleic acid sequence.

31. The synthetic nucleic acid sequence of claim 1, wherein the nucleic acid has a continuous stretch of common codons, which continuous stretch comprises at least 90% of the codons of the synthetic nucleic acid sequence.

32. The synthetic nucleic acid sequence of claim 1, wherein the nucleic acid has a continuous stretch of common codons, which continuous stretch comprises at least 95 % of the codons of the synthetic nucleic acid sequence.